lengthening). At large I_{SAC} levels causing substantial membrane depolarization ($\geq$5 mV) and inactivation of the Na^+ current, the dependence of CV on tissue deformation was blunted or even inverted, with lengthening causing conduction slowing.
Thus, during length changes of $\pm$10%, axial tissue resistance and I_{SAC} modulate conduction in opposite directions. However, at physiological I_{SAC} levels, CV is primarily determined by axial tissue resistance.

1457-Pos Board B349
Resolution of Hypo-Osmotic Stress in Isolated Mouse Ventricular Myocytes Leads to Detubulation
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It has been recently shown that various stress-inducing manipulations in isolated ventricular cardiac myocytes may lead to significant remodeling of t-tubules. Osmotic stress is one of the most common complications in various experimental and clinical settings, and therefore, this study was designed to test a hypothesis that osmotic challenge may affect the integrity of t-tubules. T-tubular remodeling in mouse ventricular myocytes in response to various osmotic challenges was studied using two approaches: (1) electrophysiologically, by measuring membrane capacitance and I_{K1} tail currents originating from K^+ accumulation in ttubules, and (2) using confocal microscopy of fluorescent dextrans trapped in vesiculated t-tubules. In particular, application and removal of 0.6 T (60% of NaCl) hypo-osmotic solution to myocytes led to ~30% reduction in membrane capacitance, ~3-fold reduction in the amplitude of I_{K1} tail current and ~2-fold reduction in so-called I_{K1} 'inactivation' (due to depletion of t-tubular K^+) at negative membrane potentials – all being consistent with strong detubulation. Importantly, confocal imaging experiments showed that extracellularly applied dextrans become trapped inside the myocytes only upon removal of hypo-osmotic solutions (i.e. during shrinking phase) but not during initial swelling period. In light of these data some relevant previous studies, including those on EC coupling phenomena during hypo-osmotic stress, may need to be reinterpreted and the experimental design of future experiments should take into account the novel findings.

1458-Pos Board B350
Fluid Pressure-Activated Non-Selective Cation Current and Cl^- Current in Rat Atrial Myocytes
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When intact atrial muscle is exposed to turbulant flow or high fluid pressure during valve diseases, it produces arrhythmias. Here we characterized a novel fluid pressure (FP)-gated ionic current (I_{FP}) in single rat atrial myocytes using a whole-cell patch clamp. A flow of pressurized (~16 dyn/cm^2) fluid was applied onto single rat atrial myocytes using a microperfusion method. The application of FP with a normal bath solution elicited a transient inward current (~1 pA/pF at $-$80 mV). The magnitude of I_{FP} was increased in a pressure-dependent manner. The removal of extracellular Ca^{2+} largely enhanced the I_{FP} and eliminated the current adaptation. Under physiological ionic gradients, the I_{FP} displayed an inwardly- and outwardly-rectifying current-voltage relationship with a reversal potential (E_{rev}) of approximately $-$52 mV. The Cl^- channel blockers, DIDS and 9-AC, suppressed inward and outward I_{FP} by about 50% and 70-80%, respectively. In symmetrical Cl^- solutions, the E_{rev} was shifted rightward ($\cong -18$ mV) and the outwardly rectifying I_{FP} was attenuated. In the symmetrical Cl^- conditions, removal of extracellular Na^+ largely reduced inward I_{FP}, and produced a left shift of E_{rev} ($\cong -64$ mV). In addition, the elimination of internal K^+ shifted E_{rev} to $\cong +8.4$ mV and decreased outward I_{FP}. Although low concentrations of extracellular Ca^{2+} blocked I_{FP} with a negative shift of E_{rev}, high concentrations of extracellular Ca^{2+} produced a right shift of E_{rev}. Gadolinium ion (Gd^{3+}), the stretch-activated channel blocker, partially blocked the inward I_{FP}. In current-clamped cells, FP of the same magnitude elicited spontaneous membrane depolarization with repetitive action potentials and prolonged action potential durations. These results indicate that FP may activate an outwardly rectifying Cl^- channel and a Gd^{3+}- and Ca^{2+}-sensitive non-selective cation channel that carries Na^+, K^+, and Ca^{2+}.

1459-Pos Board B351
Cardiac Na/K-ATPase and Na/Ca Exchange Function is Altered in Ankyrin B Heterozygous Mice
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Ankyrin-B (AnkB) is a multivalent ''adaptor'' protein that targets select membrane proteins to the cytoskeleton. Loss-of-function mutations in AnkB may cause ventricular arrhythmias and sudden cardiac death in humans. Direct interaction with AnkB is required for the membrane targeting and stability of Na/Ca exchanger (NCX) and Na/K-ATPase (NKA), key regulators of cardiac contractility and arrhythmogenesis. However, it is currently unknown how AnkB modulates NCX and NKA function. To investigate this, we used AnkB heterozygous mice ($AnkB^{+/-}$) and their wild-type (WT) littermates. Cardiac myocytes from $AnkB^{+/-}$ mice show reduced expression (by ~20%) and altered localization of both NCX and NKA. In agreement with the lower protein level, we found slower decay of the caffeine-induced Ca transient (τ=7.4$\pm$0.8 sec *vs.* 5.2$\pm$0.6 sec) and reduced maximum rate of NKA-mediated Na extrusion (5.0$\pm$0.5 *vs.* 6.4$\pm$0.4 mM/min) in intact myocytes from $AnkB^{+/-}$ mice *vs.* WT. Thus, NCX and NKA transport function are reduced in $AnkB^{+/-}$*vs.* WT mice. We also measured the voltage-dependence of the currents carried by NCX (I_{NCX}) and NKA (I_{pump}) using whole-cell voltage-clamp. I_{NCX} and I_{pump} were recorded during descending voltage ramps, as Cd-sensitive and K-activated currents, respectively. I_{NCX} reflected the lower NCX expression in $AnkB^{+/-}$ myocytes, with no difference in the voltage-dependence *vs.* WT. In contrast, I_{pump} had a significantly ($p<0.001$) steeper voltage-dependence in $AnkB^{+/-}$*vs.* WT myocytes. Thus, at $-$80 mV, close to the resting membrane potential, I_{pump} was reduced by ~35% in $AnkB^{+/-}$ mice, whereas at +30 mV, close to the peak of the action potential, $AnkB^{+/-}$ I_{pump} was elevated by ~18% *vs.* WT. Thus, in addition to reducing NKA protein expression, AnkB also directly modulates NKA function in cardiac myocytes, by reducing the voltage-dependent I_{pump} inactivation. This could significantly affect myocyte $[Na]_i$ and $[Ca]_i$ regulation.

1460-Pos Board B352
Electrophysiologic Effects of Azithromycin in Cardiomyocytes
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The widely-used macrolide antibiotic azithromycin (AZ) increases risk of cardiovascular and sudden cardiac death. Case reports indicate that AZ can cause polymorphic ventricular tachycardia in the absence and presence of QT prolongation, implying a novel proarrhythmic syndrome. We investigated the electrophysiologic effects of AZ *in vivo* and *in vitro* using mice, cardiomyocytes, and heterologously-expressed human ion channels. After implanting an ECG telemeter, conscious adult mice received intraperitoneal injection of AZ (50 mg/kg, followed in 60 min by 100 mg/kg; n=7). With both doses of AZ, heart rate declined (from 685$\pm$24 to 489$\pm$20 and 481$\pm$21 bpm, for baseline, 50 and 100 mg/kg, respectively [mean$\pm$SEM]; $P<0.001$). In addition, AZ increased the PR interval (32.7$\pm$0.9 ms to 39.4$\pm$0.7 and 39.8$\pm$0.9 ms, respectively; $P<0.001$), QRS interval (10.2$\pm$0.4 ms to 12.5$\pm$0.4 and 13.3$\pm$0.5 ms, respectively; $P<0.001$), and QT interval (37.4$\pm$4 ms to 48.0$\pm$5 and 51.2$\pm$4 ms, respectively; $P<0.01$). In spontaneously-beating HL-1 cardiomyocytes, AZ (100 μM) significantly slowed beat rate (from 215$\pm$7 to 180$\pm$7 bpm; n=14; $P<0.01$), while increasing action potential rise time (23.0$\pm$3.0 to 36.2$\pm$3.8 ms; $P<0.01$) and duration (at 90% repolarization, 118.3$\pm$8 to 137.8$\pm$8.7ms; $P<0.01$). In HEK cells stably expressing SCN5A, AZ reduced Na^+ currents (IC_{50} 110$\pm$3 μM; n=14), while similar results were obtained using mouse ventricular myocytes (IC_{50} 117$\pm$4 μM; n=6). In addition, AZ suppressed K^+ currents recorded from HEK cells expressing hERG (IC_{50} 219$\pm$21 μM; n=5) and CHO cells expressing KCNQ1 and KCNE1 (IC_{50} 184$\pm$12 μM; n=6), as well as L-type Ca^{++} current in rabbit ventricular myocytes (IC_{50} 67$\pm$4 μM; n=5). We conclude that azithromycin blocks multiple cardiac ion channels to prolong the PR, QRS, and QT interval *in vivo*, at concentrations achievable within the heart based on intracellular drug accumulation. These effects likely contribute to its novel proarrhythmic effect in humans.

1461-Pos Board B353
Enhancement of Antioxidant Defence Preserves RyR2 Function of Hyperglycemic Cardiomyocytes via Regulation of both Intracellular Zn^{2+} and Ca^{2+} Homeostasis
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Zinc exists in biological system and resting intracellular level of free Zn^{2+} ($[Zn^{2+}]_i$) can be greatly increased by thiol-reactive oxidants or high glucose and contributes to oxidant-induced alterations in EC-coupling although in cardiomyocytes. Since $[Zn^{2+}]_i$ is altering function of numerous cellular proteins, its mobilization by reactive oxygen species in diabetic heart can be likely to cause significant effects. Therefore, we aimed to investigate the role of antioxidant-defence system in preserving of cardiac ryanodine receptor